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AIR MAIL

Dear Cavalli:

Your informative letter just received.

Enclosed find an appendix with a summary of my data on the cross of 58-161 with W-585. I am pleased that we should have made so nearly the same observations on the linkage relationships of Mal and Gal. I don't see how Mal can be put on the map at all! Once I thought that it might be between B and M (which would mask any linkage either to Lac or to B_1, but this has been excluded by crosses in which both biotin and thiamine were added to the medium. M- is exceedingly stable, so that it is possible to use it alone. If Mal were between M and B_1, it should certainly interact much more strongly than it does with B_1. It cannot be to the left of B_1 as it shows a closer linkage, with BM, and does not interact strongly with B_1. If Mal is in linear order, one would have to place it not far from B_1, and assume a non-random distribution of crossing over. I would rather relate Mal peculiarities to the fact that it is almost invariably hemizygous in the heterozygotes. If the zygotes from which prototrophs are isolated are comparable (except for persistence) to the heterozygotes, then one would get an apparent linkage based on the fact that in some way Mal# is almost always lost! The same probably applies to Gal. I've just completed some reversion experiments on Gal- heterozygotes which show that Gal is also hemizygous. However, whereas the heterozygotes (from a cross involving W-583) are almost invariably Mal- (hemizygous Mal-), many of them are either Gal- or Gal#. The Gal factor of W-583 has never been observed heterozygous, and only recently I have found the first instance of a Mal heterozygote. Heterozygosity

for Mal is certainly very rare indeed, and possibly may arise from quite a different mechanism than the other aneuploid heterozygotes. I really still do not have a good story on the mechanisms of this heterozygote, but this summer fortunately, I have a good deal of assistance, and am approaching the problem on a suitably large scale. We need mostly some good information on the inheritance of Het, but so far only the A, B, and C stocks mentioned in my paper have carried it.

In your letter you refer to the possibility of confusion based on the epistasis of Gal# to Lac-. I wonder if you do not have a typographical error. If Gal# were epistatic, then the standard Lac- stocks (e.g. Y-53 or Y-87) could not be recognized as such, since they are Gal#. Gal- is "epistatic" to Lac#, in the sense that it is sometimes difficult to score a Gal-Lac# as Lac#, i.e., the Gal- weakens the lactose fermentation.

I must certainly agree with you about the difficulty of scoring Ara-. I have not used it to any extent. It seems to be almost completely linked to Gal.

I was interested in your observation about mixed prototroph colonies. I have seen them re Lac, especially by conducting the crosses on synthetic EMB lactose, where they can be seen directly as colonies with integral sectors. However, I had the impression that rather less than 1% of the colonies were mixed for Lac. On Maltose EMB, more than 10% of the (few) Mal# prototrophs also have a Mal- sector. I have thought of these as likely to be sister meiotic products, because there are distinct correlations in their Lac and V segregations which one would not expect from distinct zygotes.

On the other hand, I think there is a good prima facie case that the mixed prototrophs you get from your Hfr crosses are likely to come from several zygotes. You point out yourself the extent of microcolony formation. If the rate of recombination is high, one would have to expect that several cells from a microcolony at a given place will each participate in a fusion and produce several zygotes.

Since you give some hint of an f-1 test of Hfr, I assume that you have succeeded in recovering multiple mutant as well as prototroph recombinants with its help. As you may imagine, I am exceedingly interested in this stock, and would like very much to have the opportunity of confirming your observations. You can be sure that you will be kept fully informed of any findings, and that we will make no attempt to intrude on any area in which you would be particularly interested. If you agree to send it, I would be most appreciative. However, I hope that this request will not embarrass you, as I can well understand that you might have some reticence about distributing it so early in your investigation. I gather that you are actively pursuing the problem of the mechanism whereby Hfr is more active than 58-161 in producing prototrophs. Have you considered the likelihood that it may be much more motile? I have often thought that their might be a chemotropism between distinct mutants, for each produces the needs of the other, and some such phenomenon might dispose of the kinetic difficulties.

Lately, I have realized that some of my stocks are consistently Lfr (low frequency of recombination) for no obvious biochemical reason. This has been particularly true of crosses of Lac_2^- with Lac_1^-, even on glucose medium on which either of them grow very well with proper supplements. I have also noticed this among a number of segregants from H- stocks, again with no obvious chemical basis. I have hoped to use these Lfr stocks to explore environmental conditions which might stimulate recombination, thinking that the "standard" rate was nearly all one could expect on kinetic grounds. I will be glad to send you some of these if they would help your analysis.

Secondly, one of the most promising leads in the investigation of the heterozygotes has been the finding that some segregants give markedly altered ratios of Lac# and Mal# prototrophs (both greatly increased) compared to biochemically comparable stocks. This tends to support the notion that a chromosomal aberration is involved, but is still far from definitive.

With all of the confusion concerning the linkages of Gal and Mal, I wonder whether you may not be very sceptical about linearity. Certainly I would be! However, may I suggest that you sometime try the V_6-Lac-V_1 series mentioned in my Genetics paper which gave quite cleancut results.

Yours very sincerely,

Joshua Lederberg

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P.S. On looking over my notes, I find that I cannot give you any worthwhile account of the crosses I did with 58-161 x W583. I noticed that there were very few Gal# Lac- or Gal- Lac#, but decided that I couldn't score the latter at all. Later I noticed that 3% lactose facilitated scoring, but I haven't used this to collect quantitative data. However, here are some scores for maltose and galactose.

M-G-	M-G#	M#G-	M#G#		
34	10	5	2	T (0)	Exp. 358
43	15	2	0	T (B_1)	

When I realized that G-L# was difficult to score, I abandoned W583 and used W677 which has the other disadvantage that Gal itself is difficult to read. I am trying to perfect better stocks. These data certainly put Gal and Mal rather far apart, and I still wonder whether Gal isn't near Lac, as you indicate. Your data show about 7% triples. This is disturbingly high (like table 6 in my Genetics paper) but it seems to be so!